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(54) **PORCINE REPRODUCTIVE RESPIRATORY SYNDROME (PRRS) VACCINE AND DIAGNOSTIC.**

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Description

The present invention is concerned with a vaccine for the protection of pigs against Porcine Reproductive Respiratory Syndrome (PRRS), a biological composition comprising viruses of a novel virus type, a method for the preparation of viral antigen derived from viruses of this novel virus type, a method for the detection of antibodies to PRRS-virus, as well as a diagnostic test kit to be used in this method.

A new porcine disease has attacked over 5.000 North European pig farms since late 1990. This disease is now called Porcine Reproductive Respiratory Syndrome (PRRS). First, identified in Germany in December of 1990, the problem became increasingly critical in the beginning of 1991. In January and February of 1991 the disease spreaded to The Netherlands and Belgium. Outbreaks have also been reported from Spain.

It is anticipated that the disease will become very costly from an economic standpoint, comparable or even worse than Aujeszky's Disease.

The principal clinical signs in sows are anorexia and late abortion up to day 110 of pregnancy. With piglets a high incidence of stillborn weak piglets in addition to respiratory problems are observed. In fatteners chronic pneumonia and increased mortality occurs.

In order to develop a vaccine to protect pigs against PRRS or to develop a diagnostic method to determine infection in pigs, the causative agent of this disease has to be identified. However, up to now only a few characteristics of the causative agent have been disclosed, such as its viral nature, haemagglutination properties, buoyant density and growth characteristics in vitro (Wensvoort, G. et al., Vet. Quaterly 13, 121-130, 1991).

International patent applications WO 92/21375, WO 93/06211, WO 93/03760 and European patent application EP 0529584 (Boehringer Ingelheim Animal Health Inc.) are conflicting applications available as prior art for the present invention under Article 54(3) and (4) EPC. WO 92/21375 (Stichting Centraal Diergeneeskundig Instituut) discloses the identification of the causative agent, called Lelystad Agent, of the Mystery Swine Disease (MSD). Additionally, the use of this agent for the preparation of vaccines and diagnostic tests is proposed. Both WO 93/03760 (Collins et al.) and EP 0529584 disclose the isolation of an US isolate, i.e. isolate ATCC-VR 2332, and identify this isolate as the causative agent of MSD in the US. The preparation of an attenuated MSD vaccine by passaging the isolate on a cell culture is also disclosed therein. WO 93/06211 (Collins et al.) describe the isolation of an infectious tissue homogenate from a diseased animal. However, no identification of the causative agent present in the homogenate could be given.

It is an object of the present invention to provide sufficient and unequivocally identifying characteristics of the new causative agent of PRRS necessary in order to prepare a vaccine and diagnostic test for the causative agent.

We now have identified a novel virus type, called PRRS virus, responsible for this disease, the novel virus type being characterized by the virus deposited at the CNCM under accession no. I-1140.

The virus of the new type can be isolated from lung tissues of clinical cases of PRRS. A 24-hour monolayer of alveolar macrophages was incubated with a 50% homogenate of lung tissue in phosphate buffered saline (PBS). The macrophage cell culture was established from lung lavages of SPF piglets with PBS. The macrophages were washed and incubated with RPM 1640 medium supplemented with 10% fetal calf serum and antibiotics for 24 hours in 5% CO_2 incubator.

A small volume of the homogenate is added to the macrophages, after incubation for 1 h. at 37 °C new medium was added again and the infected macrophages were further incubated at 37 °C in CO_2 atmosphere. Total cytophatic effect (CPE), is apparent after 2-3 days of incubation.

The virus can also be isolated from other sources such as heart, tonsils, brain and liver of infected pigs.

The selected tissue homogenates of a clinical case of PRRS used for the isolation of the novel PRRS virus did not show consistently signs of the presence of other viral agents commonly found with pigs, especially not pseudorabies virus (PRV), porcine parvovirus (PPV), paramyxovirus, hog cholera virus (HCV) and transmissable gastroenteritis virus.

Other characteristic features of the novel PRRS virus are outlined below:

- virus deposit

The novel PRRS virus specifically isolated as described above was accorded the internal description "Isolate no. 10" and has been deposited September 6, 1991 with the Collection Nationale de Cultures de Micro-organismes (CNCM) of the Institut Pasteur at Paris, France under the accession number I-1140.

- growth characteristics

The novel virus grows on macrophages but does not grow to measurable levels in the established cell lines BHK21, Vero, SK-6 (swine kidney), PK15 (porcine kidney), ST (swine testis), L929 and BEL (bovine embryo lung).

- Serological exclusion of known agents.

Using established techniques it was excluded that one of the following agents was the causative agent of PRRS.

	test methods			
agent	VN[a]	gI Elisa[b]	HI[c]	IFT[d]
pseudorabies virus	-	-	N.D.	N.D.
porcine parvovirus	-	N.D.	-	N.D.
porcine paramyxovirus	-	N.D.	-	N.D.
porcine circovirus	N.D.	N.D.	N.D.	-
chlamydia	N.D.	N.D.	N.D.	-
N.D. = not determined				

[a] = virus neutralization test (VN) as described in Virologische Arbeitsmethoden Vol. 2, Eds.: A. Mayr et al., Fisher Verlag Jena, 1977, p. 457-535;
[b] = gI Elisa test as described in manual provided with Intertest Aujeszky gI Elisa kit;
[c] = haemagglutination inhibition test (HI) Virology, a practical approach, Ed.: B.W.J. Mahy, IRL Press Oxford, 1985, p. 245-248;
[d] = immunofluorescence test; Virologische Praxis, Ed.: G. Starke, Fischer Verlag Jena, 1968, p. 227-241.

- Serological correlation of the infectious agent with PRRS.

Field serum samples obtained from clinical cases were tested in an immunofluorescence test (IFT) using infected macrophages.
Further serum samples from pigs experimentally infected with the new virus were tested.
Microtitre plates seeded with Alveolar macrophages (4.10^4 per well) were infected using Isolate No. 10 and incubated for 24 hours. At this time first signs of CPE appear. The cells are fixed with cold 96% ethanol (30 min.). Serial dilutions starting from 1/10 of the serum samples were incubated on the fixed infected macrophages for 1 hour at 37 °C. The second incubation was with rabbit anti-pig IgG conjugated to FITC (Nordic, Breda) (1/40 dil., 30 min., 37 °C). With an inverted Leitz immunofluorescence microscope specific fluorescence was determined. Specific fluorescence is characterized by siccle shaped perinuclear fluorescence.
In the Table below a comparison of a test set of sera is given.

serum	IFT
1. Experimental infection preserum	-
2. Experimental infection one month post-infection	+
3. Field serum 1	+
4. Field serum 2	+
5. Field serum 3	+
6. Field serum 4	+
7. Field serum 5	+

- Cross-reaction between PRRS isolates.

Although, the PRRS virus is not antigenically related to known porcine viruses it could be demonstrated that different isolates of PRRS viruses are antigenically similar as they immunologically cross-react in the IF test.
The table below demonstrates that PRRS positive serum reacts with the various PRRS isolates in the IFT. Thus, any PRRS virus isolate can be used in the present invention because of their strong antigenically relationship as demonstrated with the IFT.

isolate	IFT pos. serum
Intervet 10	+ + +
Intervet 2	+
Intervet 3	+ +
Intervet 4	+ +
Geld B. 2	+ + +
Geld B. 3	+ +

- Other characteristics

The PRRS virus is an envelopped virus (sensitive for chloroform and ether treatment) with small knots on the surface and is similar to Lactate Dehydrogenase Virus and Equine Arteritis Virus. The virus particle is about 65 nm and has a buoyant density of 1.1 g/cm^3.
In macrophage cell culture infected with the virus at least two virus-specific proteins can be detected of about 14.000 and 21.000 (Da).

In accordance with the present invention, a vaccine is provided for the protection of pigs against PRRS, comprising viral antigen derived from viruses of a novel virus type characterized by the virus deposited at CNCM under accession No. I-1140.

From the above it is clear that the present vaccine can be prepared from both the Isolate no. 10 deposited with the CNCM under no 1-1140 and from any other available or isolatable PRRS VIRUS isolates immunologically related thereto.

Preferably, a vaccine is provided using as viral antigen PRRS virus which is at least partly deprived of its pathogenic properties without loosing its antigenic activity, i.e. the ability to stimulate the porcine immune system. This may be effected by attenuation or by inactivation of the virus in which latter case the virus also looses its ability to multiply.

In particular, the present invention provides an inactivated vaccine comprising one or more isolates of the novel virus in an inactivated form.

Preferably, the inactivated vaccine according to the invention comprises an amount of PRRS virus which is the equivalent of a pre-inactivation virus titre of greater than about $10^{6.5}$ $TCID_{50}$/ml (dose) and more preferably greater than about $10^{7.5}$ $TCID_{50}$/ml (dose) as measured with the method described in A. Mayr et al. (Eds.), Virologische Arbeitsmethoden Vol. 1, Fischer Verlag Jena, 1974, p. 36-39.

Inactivated PRRS fluids may also be concentrated by any number of available techniques such as an Amicon concentrating device, precipitation techniques such as with ammoniumchloride or polyethylene glycol, concentration with Carbowax$^{(R)}$ or by means of ultra-centrifugation, or adjuvant concentration techniques such as with aluminium phosphate.

The aim of inactivation of the PRRS viruses is to eliminate reproduction of the viruses. In general, this can be achieved by chemical or physical means. Chemical inactivation can be effected by treating the viruses with, for example, formaldehyde, β-propiolactone, ethylene-imine or a derivative thereof, an organic solvent (such as Tween(R), Triton X(R), sodium desoxy-cholate, sulphobetain or octyl trimethylammonium salts). If necessary, the inactivating compound is neutralized afterwards; material inactivated with formaldehyde can, for example, be neutralized with metabisulphite. Physical inactivation can preferably be carried out by subjecting the viruses to energy-rich radiation, such as UV light, X-radiation or γ-radiation. If desired, the pH can be brought back to a value of about 7 after treatment.

Usually, an adjuvant and if desired one or more emulsifiers such as Tween(R) and Span(R) are also incorporated into the inactivated vaccine. Suitable adjuvants are for example vitamin-E acetate o/w emulsion, aluminium hydroxide, -phosphate or -oxide, (mineral) oil emulsions such as Bayol(R) and Marcol 52(R) and saponins.

For the production of the live attenuated virus vaccine according to the invention, a number of methods known in the art for this purpose are available, e.g. adaption of a specific PRRS virus isolate to growth in embryonated eggs or to a culture containing susceptible porcine tissue cells or other susceptible tissue cells, and attenuation for example by 10-200 passages in eggs or such cultures, after which the virus is multiplied and harvested by collecting egg material or the tissue cell culture fluids and/or cells. Optionally, during harvesting the yield of the viruses can be promoted by techniques which improve the liberation of the infective particles from the growth substrate, e.g. sonication.

It is advantageous to add a stabilizer to the live viruses, particularly if a dry composition is prepared by lyophilization. Suitable stabilisers are, for example, SPGA (Bovarnik et al., J. Bacteriology 59, 509, 1950), carbohydrates (such as sorbitol, mannitol, trehalose, starch, sucrose, dextran or glucose), proteins (such as albumin or casein), or degradation products thereof, and buffers (such as alkali metal phosphates). If desired, one or more compounds with adjuvant activity as described above can also be added.

For live vaccines the dose rate may range from $10^{1.0}$ - $10^{7.0}$ $TCID_{50}$ per pig.

A vaccine according to the invention may be administered by intramuscular or subcutaneous injection or via intranasal, intratracheal, oral, cutane, percutane or intracutane administration.

The vaccine according to the invention can be administered to pigs depending on the vaccination history of the sows at 1, 5 or 10 weeks of age, to sows before mating and/or 4-6 weeks before farrowing (booster vaccination), or to boars each half a year (boosters).

Vaccines according to the present invention, preferably the vaccines containing inactivated PRRS viruses may contain combinations of the PRRS component and one or more unrelated porcine pathogens, such as Actinobacillus pleuropneumonia, Pseudorabies virus, Porcine influenza virus, Porcine parvo virus, Transmissible gastro-enteritis virus, rotavirus, E. coli, Erysipelo rhusiopathiae, Pasteurella multocida, Bordetella bronchiseptica.

Although a vaccine according to the invention may be derived from any PRRS virus isolate immunologically related to PRRS Isolate no. 10, preferably, the vaccine is derived from PRRS Isolate no. 10, CNCM accession no. I-1140.

A method for the preparation of PRRS viral antigen useful to be incorporated into a vaccine according to the invention includes the steps of

a) inoculating susceptible tissue culture cells with the PRRS virus,

b) cultivating the cells, and

c) harvesting the viral antigen from the culture.

Preferably, the PRRS virus is cultured to high titres, i.e. at least $10^{6.0}$ $TCID_{50}$/ml.

In particular, the PRRS virus Isolate no. 10, CNCM accession no. I-1140 is used for the preparation high amounts of PRRS viral antigen.

Viral antigen to be incorporated into a vaccine according to the invention can be prepared by the growth of the PRRS virus isolates on macrophages (alveolar, peritoneal, peripheral, bone marrow or from any other site). Also other cells with macrophage-like properties are usefull, like promonocytes, monocytes, brain vascular endothelial cells and microglial cells The macrophage or macrophage-like cells may be of spf or non-spf origin (e.g. from regular commercial pigs). In the latter cases pre-cautions are taken for undesired contamination, e.g. by the use of proper antibiotics in the culture.

Another set of routes which can lead to a very useful procedure of virus growth is the establishment of a macrophage cell line. A number of possibilities which can lead to such desirable macrophage cell lines are outlined below.

Immortalisation of macrophages using conditioned medium of L(929) cells, SK6, ST, Vero or BHK cells. In these conditioned media at least the presence of lymphokines like the macrophage Colony Stimulating Factor (CSF) is of importance (Stanley, E.R., Methods Enzymology 116, 564-587, 1985).

Treatment of macrophages of any source with chemicals to invoke immortalization, e.g. β-propiolactone as an example of an alkylating agent that effects DNA metabolism can be used (Logrippo, G.A. and Harman, F.W., J. Immunol. 75, 123-128, 1955).

A more defined way of immortalisation is the use of transforming viruses. Not limited to but especially retroviruses (e.g. leukemia virus), SV40 and Papilloma viruses are thusfar described to immortalise macrophages of other species like man and mouse. (Robinson, D.H. et al., Blood 77, 294-305, 1991; Righi, M. et al., Oncogene 6, 103-111, 1991; Choi, C.S. et al., Arch. Virol. 115, 227-237, 1990; Gendelman, H.E. et al., Lab. Invert. 51, 547-555, 1984; ATCC catalogue for available mouse and human macrophage lines).

An even more precise way of immortalisation is the use of the genes of any of above mentioned or other viruses that are responsible for the immortalisation, for example Vmyc or Vmfs genes and/or the large T gene of SV40 may be used. The construction of a retroviral vector that allows the integration of a transforming gene like SV40 large T into the genome of the host cell would be the most preferable solution in that immortalisation is obtained without the actual replication and excretion of viral or viral-like particles. Also a plasmid containing a selectable marker (e.g. Neo$^{(R)}$) and the SV40 large T gene would be a method for immortalisation.

As a source for immortalisation mature macrophages can be taken, however, very useful are also the cell stages before the differentiation to the mature macrophages because these are dividing cells with macrophage characteristics, and have macrophage markers as CD1, CD11 and CD14. The pre-stages can be selected and obtained in pure form free from contamination by other lymphocytes using various separating techniques for-instance cell sorting with a FACS (Fluorescence Activated Cell Sorter) and fluorochrome labeled specific antibodies against the CD markers.

The demand for sensitive, specific methods for screening and identifying carriers of PRRS virus is significant.

Therefore, the present invention is also directed to a rapid and sensitive assay for the detection of antibodies to PRRS virus.

It is a further object of the invention to provide diagnostic test kits for performing the assay method of the invention.

According to the present invention a method for the detection of antibodies to PRRS virus is provided comprising:

a) incubating a sample suspected of containing anti-PRRS virus antibodies with PRRS viral antigen reagent derived from viruses of a novel virus type characterized by the virus deposited at the CNCM under accession no. I-1140, under conditions which allow the formation of an antibody-antigen complex, and

b) detecting the antibody-antigen complex.

The viral antigen from PRRS infected cells which react immunologically with serum containing anti-PRRS virus antibodies are useful as reagent in immunoassays to detect presence of PRRS virus antibodies in biological samples. The viral antigen reagent can be, inter alia, purified or partially purified virus particles or viral polypeptides, disrupted virus or infected cells or cell lysate therefrom.

The design of the immunoassay may vary. For example, the immunoassay may be based upon competition or direct reaction, or sandwich type assays. Furthermore, protocols may use solid supports or may be by immuno precipitation. The detection of the antibody-antigen complex may involve the use of labeled antibody; the labels may be, for example fluorescent, chemiluminescent, radio-active, dye molecules or enzyme labels.

Present methods useful for the detection of antibodies to PRRS virus include the enzyme-linked immuno sorbent assay (ELISA), immunofluorescence test (IFT) and Western Blot technique.

The ELISA technique involves the reacting of a test sample with viral antigen reagent obtained from PRRS virus. Preferably, the antigen reagent is a cell lysate of macrophages infected with PRRS virus. Typically, the antigen reagent is coated onto a solid phase, e.g. a microplate or plastic cup. After washing to remove the unbound antibodies anti-immunoglobulin labeled with enzyme is added to the solid phase, incubated, and washed again. Enzymes suitable for labeling are known in the art and include for example horseradish peroxidase. Thereafter, an enzyme substrate is added to the mixture and a detectable, measurable color product is formed in the presence of antibodies to PRRS virus.

In addition, the IFT technique can be used according to the present invention. For example, macrophages infected with PRRS virus are cultured in microtitre plates for 24 hours. Thereafter the cells are fixed with for example acetone or ethanol or by freeze-thawing in combination with formaline treatment. The test sample is then added to the fixed cells and incubated for 1 h. at 37 °C. Subsequently, antiimmunoglobulin conjugated with a fluorescent compound such as fluorescein is added to the fixed cells. Reaction mixtures are then examined for fluorescence under a microscope. A typical fluorescence pattern of PRRS virus

infected macrophages incubated with a positive serum of a pig infected with PRRS can de demonstrated.

The preferred diagnostic method according to the invention is directed to the detection of PRRS antibodies using the Western Blot technique.

In this technique PRRS viral antigen is electrophoretically resolved on SDS-polyacrylamide gels. Preferably, the viral antigen is the cell-lysate of PRRS virus infected macrophages. The resulting protein bands are electro-transfered, preferably to nitrocellulose paper. Other types of paper, known to those skilled in the art, such as diazo paper are also suitable. (Tsang et al., In: Methods in Enzymology 92, chapter 29, 1983). The nitrocellulose strips containing the resolved viral antigen are then incubated with the test samples, and if desired with positive and negative reference samples. If desired negative reference strips, e.g. containing electrophoretically resolved macrophage lysate obtained from macrophages not-infected with PRRS virus are also incubated with the test sample. The positive reference sample is typically a sample known to contain antibodies to PRRS virus, e.g. serum from clinical cases of PRRS or from animals experimentally infected with PRRS virus. The negative reference sample is a sample known to be devoid of antibodies to PRRS virus. Detection of the antibody-antigen complex may then be carried out by either ELISA or solid phase strip radio-immunoassay techniques. Washings may be carried out after each incubation.

Preferably, the incubation of the strips is carried out in containers.

Surprisingly, the Western Blot technique is particularly suitable for the detection of PRRS virus specific polypeptides obtained from an in vitro cell culture because of the presence of two or three small virus specific proteins separately identifiable from other material reactive with PRRS serum. As is shown in Figure 1 the major immune reactivity or specificity is directed against an about 14.000 and 21.000 (Da) protein of the PRRS virus (Western blot of glycerol gradient purified PRRS virus cultured on macrophages) The 21.000 (Da) band is difuse and therefore the designation 21.000 (Da) is only meant as an indication of the size of this protein.

Other virus specific proteins recognized by antibodies to PRRS virus in Western blot have molecular weights of about 46.000, 49.000 and 55.000 (Da).

With purified ^{35}S-Met/Cys labeled virions, 14K, 24K, 33K and 46K proteins were shown to be prominent. Radioimmuno precipitation of ^{35}S-Met/Cys labeled infected cell lysates resulted in demonstration of two virus-specific proteins of about 24K and 33K. The above-noted virus specific proteins can also be used for the, diagnosis of PRRS infection.

In a preferred embodiment of the invention the purified 14.000 and/or 21.000 (Da) PRRS virus protein may be used in the ELISA and Western Blot technique as the viral antigen reagent in order to provide a more sensitive assay.

In accordance with an other embodiment of the invention, a diagnostic test kit is provided which permits "on site" screening for antibodies to PRRS virus. The test kit includes at least one strip containing resolved PRRS viral antigen, (electro-) transfered from a SDS-PAGE gel. In addition the kit may comprise a negative control reference strip, e.g. containing resolved antigen from an in vitro cell culture, e.g. macrophage cell culture, not-infected with PRRS virus.

Preferably, the strips are contained in individual containers facilitating the subsequent incubation steps.

Furthermore, it is advantageous to include in the test kit the positive and negative reference samples in order to facilitate evaluation of the test results by comparison with the results obtained for the test sample.

If desired pre-developed positive and negative reference strips are also provided in the kit. The pre-developed strips are used to evaluate the test results by a visual comparison with the test strips after condition of a color reaction. The pre-developed strips facilitate reading the assay results and practically eliminate the need for a skilled technician to evaluate the results.

Included in the kit may also be vials of enzyme-conjugated antiserum reagent, preferably horseradish peroxidase conjugated immunoglobulins, substrate or color change indicator, washing buffers and solution for terminating the color reaction. The preferred substrate, reaction terminating agent and washing buffers are DAB, distilled water and PBS Tween(R) and PBS, respectively.

In a most preferred embodiment of this test kit the about 14.000 and 21.000 (Da) protein bands of the PRRS virus test strips containing the resolved PRRS viral antigen are separately identifiable, i.e. these bands are separated from other viral or non-viral antigens reactive with the PRRS serum.

To measure PRRS viral antigen in a test sample, known PRRS specific antiserum or antibodies are used as a reagent, preferably fixed to a solid phase. The test sample containing antigen is added, and after incubation allowing the formation of an antigen-antibody complex, the solid phase is washed, and a second labeled antibody is added to detect the antigen-antibody complex.

Furthermore, the present invention is directed to test kits to be used in accordance with the method for the detection of PRRS viral antigen in a test sample as described above.

Example 1

Isolation and propagation of PRRS virus

SPF piglets kept in isolation facilities are aneasthesised and lung lavage is performed using warm phosphate-buffered saline (PBS). About 100 ml of macrophages suspension is collected in siliconised glass bottles. The macrophages are washed with PBS and incubated with RPM 1640 medium (Flow Labs) supplemented with 10% fetal calf serum and antibiotics for 24 hours in 5% CO_2 - incubator. The cells were seeded in 25 cm^2 Roux flasks (Falcon) at a density of 3 x 10^5 per cm^2.

Sow 266 suffering from clinical symptoms of PRRS (abortion, off feed) diagnosed by a veterinarian of the Regional Animal Health Institute was taken for post mortem.

A 50% homogenate of lung tissue in PBS was prepared using an Ultraturrax(R). Low speed centrifugation was used to clarify the suspension. One ml of the clarified supernatant was taken and inoculated onto a 24-hour monolayer of alveolar macrophages. After incubation for 1 h at 37 °C the medium was added again and the infected macrophages were further incubated at 37 °C in CO_2 atmosphere. After 2 days first signs of CPE became apparent. At day 3 the macrophages showed complete lysis, which was confirmed by the uptake of trypan blue.

Viable cells do exclude trypan blue dye, as did the non-infected control macrophages. The harvest of this first passage was stored at -70 °C. Further passages of this isolate were made by incubation of 1 ml prediluted 1:10 - 1:100 virus on further macrophages cultures. Total CPE is apparent after 2-3 days of incubation. This PRRS isolate was accorded the internal code "Isolate No. 10". A sample of this isolate has been deposited with the CNCM under accession No. I-1140.

The method for the preparation of PRRS viral antigen as described above results in the ability to grow the virus to high titres in vitro.

Table 1 shows the results of the virus yield after several passage levels of Isolate No. 10.

Table 1

Growth characteristics in macrophages	
passage level	Titre ($TCID_{50}$/ml)
3	$10^{6.0}$
4	$10^{6.0}$
5	$10^{7.0}$
6	$10^{6.7}$
7	$10^{7.4}$
8	$10^{7.5}$

Example 2

Experimental infection of PRRS virus in four-week-old pigs

Isolate No. 10 ($10^{5.0}$ $TCID_{50}$) was inoculated intranasally into two four-week-old pigs housed in one pen with a contact control. Clinically in the animals anorexia was observed for four days and a slight transient temperature rise. Further for 6 days they were duff and slow. 8 Days p.i. the two infected animals were anaesthetised and lung lavages were performed. In one of those pigs the causative agent was reisolated upon co-cultivation with spf macrophages.

Furthermore seroconversion was measured in both the infected pigs as well as in the contact control.

Seroconversion was measured in the Immuno-Fluorescent Assay (IFA). Monolayers of macrophages 24 hours after infection with Isolate 10 were fixed with 95% -70 °C ethanol for 1 hour and incubated with the test sera for 1 hour 37 °C. FITC-conjugated rabbit anti-swine IgG was added and incubated for 30 min at 37 °C. The samples were read using an UV-fluorescent microscope (Leitz). 4 weeks after vaccination antibody titer in the vaccinates was more than 1280.

Example 3

Vaccination of pregnant sows

5 Sows 10 weeks from time of farrowing were housed in containment facilities. 3 Sows are vaccinated i.m. (pre-inactivation titer $10^{7.2}$ $TCID_{50}$/ml) in the neck with inactivated isolate No. 10 adjuvanted in a water-in-oil (Freund incomplete) emulsion (1 ml) and boostered 4 weeks later. At day 80 of gestation, about 3 weeks before delivery, the vaccinates and controls are challenged infected intranasally with Isolate No. 10 ($10^{5.0}$ $TCID_{50}$).

The serological response in the sows is monitored.

The challenge of the vaccinated sows before farrowing revealed a protection. The vaccinated sows had significantly more healthy piglets than the controls (Table 2). The table shows the effect of the challenge on the progeny of vaccinated and control sows at the time of birth and for the live born during the first week after birth.

Table 2

Treatment	Piglets born[a]		Neonatal piglets[a]		
	Mummies	Deaths	Deaths	Weak	Healthy
Vaccinated	1.7	2	1.7	0.7	6.7
Controls	1	3	3	2	2

[a] mean number of piglets per sow.

Example 4

Western Blot immuno-assay

Supernatants from infected as well as uninfected macrophage cultures were clarified at 3.000 g. The supernatants were centrifuged in a SW 28.1 rotor at 25.000 rpm for 3 hours. The pellets were resuspended in TEN-buffer and used in gel electrophoresis.

The material was separated by electrophoresis under reducing conditions in a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel. The gels were equilibrated in renaturation buffer (4 M urea, 50 mM NaCl, 10 mM Tris hydrochloride, 0.1 mM dithiothreitol [pH 7]) for 30 min. and then incubated in electrophoresis buffer without SDS for an additional 30 min. Electrophoretic transfer to nitrocellulose membranes (Schleicher & Schuell, Dassel, FRG) was performed in a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad, Munich, FRG) at 2 A for 1 h. Nonspecific binding of proteins to the membrane was blocked by incubation with dry milk powder solution in PBS for 2 h. PRRSV-positive pig antisera diluted in dry milk powder solution in PBS were allowed to bind overnight, and specific binding was detected using a goat anti-porcine peroxidase-conjugated antiserum (Dianova) and 2,2-chloronaphthol (Sigma) as the substrate.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE,

1. A vaccine for the protection of pigs against Porcine Reproductive Respiratory Syndrome (PRRS) comprising viral antigen derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, wherein the viral antigen is inactivated PRRS virus and the vaccine comprises:
   a. an amount of the inactivated viral antigen which is equivalent to a pre-inactivation virus titre of at least $10^{6.5}$ $TCID_{50}$/ml, preferably at least $10^{7.5}$ $TCID_{50}$/ml, or
   b. an adjuvant selected from the group consisting of vitamin-E acetate o/w-emulsion, aluminium phosphate, - oxide, an oil-emulsion provided that the oil-emulsion is not Freund's adjuvant, and saponins, or
   c. the PRRS virus deposited at the CNCM under accession no. I-1140.

2. A vaccine for the protection of pigs against Porcine Reproductive Respiratory Syndrome (PRRS) comprising viral antigen derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, wherein the viral antigen is a live PRRS virus and the vaccine comprises:
   a. the PRRS virus in a lyophilized form, or
   b. a stabilizer, or
   c. an amount of $10^{1.0}$-$10^{7.0}$ $TCID_{50}$ of the PRRS virus per dose, or
   d. an adjuvant selected from the group consisting of vitamin-E acetate o/w-emulsion, aluminium phosphate, - oxide, an oil-emulsion and saponins, or
   e. the PRRS virus deposited at the CNCM under accession no. I-1140.

3. A vaccine for the protection of pigs against Porcine Reproductive Respiratory Syndrome (PRRS) comprising more than one antigen component, characterized in that a first component comprises the PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, and a further antigen component which is derived from an unrelated porcine pathogen.

4. A PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, which virus can be propagated in cell culture to a titre of at least $10^{6.0}$ $TCID_{50}$/ml, and preferably at least $10^{7.0}$ $TCID_{50}$/ml.

5. A biological composition comprising the PRRS virus according to claim 4.

6. A method for the preparation of a live attenuated vaccine for the protection of pigs against PRRS comprising the steps of:
   a. passaging a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, on a susceptible substrate 10-200 times,
   b. multiplying the attenuated virus on a susceptible substrate, and
   c. collecting the virus and processing the virus into a vaccine preparation.

7. A method for the preparation of an inactivated vaccine for the protection of pigs against PRRS comprising the step of inactivating a cultured PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, with an inactivating agent selected from the group consisting of β-propiolactone, ethylene-imine or a derivative thereof an organic solvent, X-radiation and γ-radiation.

8. A method for the preparation of a viral antigen derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolates immunologically related thereto, comprising the steps of:
   a. inoculating susceptible tissue cells with the virus,
   b. cultivating the cells, and
   c. harvesting the viral antigen from the culture, wherein the pre-harvest titer is at least $10^{6.0}$ $TCID_{50}$/ml.

9. A method for the detection of antibodies to PRRS virus comprising the steps of:
   a. incubating a test sample suspected of containing anti-PRRS virus antibodies with PRRS viral antigen reagent derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, under conditions which allow the formation of an antibody-antigen complex, and
   b. detecting the antibody-antigen complex involving the use of a labeled antibody, the label being selected from the group consisting of fluorescent labels, chemiluminescent labels, radioactive labels, dye molecule labels and enzyme labels provided that the enzyme label is not used in an immuno-peroxidasemonolayer assay (IPMA).

10. A method according to claim 9, characterized in that the viral antigen reagent is a viral polypeptide.

11. A method according to claim 9, characterized in that the viral antigen reagent is a virus particle.

12. A method according to claim 9, characterized in that the method is an enzyme-linked immuno sorbent assay (ELISA).

13. A method according to claim 9, characterized in that the method is an immunofluorescence test (IFT).

14. A method for the detection of PRRS viral antigen in a test sample comprising the steps of:
    a. incubating the test sample with antibodies specific for a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, under conditions which allow the formation of an antigen-antibody complex, said antibodies being fixed to a solid phase,
    b. washing the solid phase,
    c. adding a labeled antibody, and
    d. detecting the antigen-antibody complex.

Claims for the following Contracting States : ES, GR

1. A method for the preparation of a vaccine for the protection of pigs against Porcine Reproductive Respiratory Syndrome (PRRS) comprising viral antigen derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, wherein the viral antigen is inactivated PRRS virus and the vaccine comprises:
    a. an amount of the inactivated viral antigen which is equivalent to a pre-inactivation virus titre of at least $10^{6.5}$ $TCID_{50}$/ml, preferably at least $10^{7.5}$ $TCID_{50}$/ml, or
    b. an adjuvant selected from the group consisting of vitamin-E acetate o/w-emulsion, aluminium phosphate, - oxide, an oil-emulsion provided that the oil-emulsion is not Freund's adjuvant, and saponins, or
    c. the PRRS virus deposited at the CNCM under accession no. I-1140,
    and wherein the viral antigen is mixed with a pharmaceutical acceptable carrier.

2. A method for the preparation of a vaccine for the protection of pigs against Porcine Reproductive Respiratory Syndrome (PRRS) comprising viral antigen derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, wherein the viral antigen is a live PRRS virus and the vaccine comprises:
    a. the PRRS virus in a lyophilized form, or
    b. a stabilizer, or
    c. an amount of $10^{1.0}$-$10^{7.0}$ $TCID_{50}$ of the PRRS virus per dose, or
    d. an adjuvant selected from the group consisting of vitamin-E acetate o/w-emulsion, aluminium phosphate, - oxide, an oil-emulsion and saponins, or
    e. the PRRS virus deposited at the CNCM under accession no. I-1140,
    and wherein the viral antigen is mixed with a pharmaceutical acceptable carrier.

3. A method for the preparation of a vaccine for the protection of pigs against Porcine Reproductive Respiratory Syndrome (PRRS) comprising more than one antigen component, characterized in that a first component comprises the PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, and a further antigen component which is derived from an unrelated porcine pathogen, wherein the antigen components are mixed with a pharmaceutical acceptable carrier.

4. A method for the preparation of a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, which virus can be propagated in cell culture to a titre of at least $10^{6.0}$ $TCID_{50}$/ml, and preferably at least $10^{7.0}$ $TCID_{50}$/ml, wherein a cell culture is infected with the virus and the virus is harvested from the culture after the propagation.

5. A method for the preparation of a live attenuated vaccine for the protection of pigs against PRRS comprising the steps of:
    a. passaging a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, on a susceptible substrate 10-200 times
    b. multiplying the attenuated virus on a susceptible substrate, and
    c. collecting the virus and processing the virus into a vaccine preparation.

6. A method for the preparation of an inactivated vaccine for the protection of pigs against PRRS comprising the step of inactivating a cultured PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, with an inactivating agent selected from the group consisting of β-propiolactone, ethylene-imine or a derivative thereof an organic solvent, X-radiation and γ-radiation.

7. A method for the preparation of a viral antigen derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolates immunologically related thereto, comprising the steps of:
   a. inoculating susceptible tissue cells with the virus,
   b. cultivating the cells, and
   c. harvesting the viral antigen from the culture, wherein the pre-harvest titer is at least $10^{6.0}$ $TCID_{50}$/ml.

8. A method for the detection of antibodies to PRRS virus comprising the steps of:
   a. incubating a test sample suspected of containing anti-PRRS virus antibodies with PRRS viral antigen reagent derived from a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, under conditions which allow the formation of an antibody-antigen complex, and
   b. detecting the antibody-antigen complex involving the use of a labeled antibody, the label being selected from the group consisting of fluorescent labels, chemiluminescent labels, radioactive labels, dye molecule labels and enzyme labels provided that the enzyme label is not used in an immuno-peroxidasemonolayer assay (IPMA).

9. A method according to claim 8, characterized in that the viral antigen reagent is a viral polypeptide.

10. A method according to claim 8, characterized in that the viral antigen reagent is a virus particle.

11. A method according to claim 8, characterized in that the method is an enzyme-linked immuno sorbent assay (ELISA).

12. A method according to claim 8, characterized in that the method is an immunofluorescence test (IFT).

13. A method for the detection of PRRS viral antigen in a test sample comprising the steps of:
   a. incubating the test sample with antibodies specific for a PRRS virus deposited at the CNCM under accession no. I-1140 or a PRRS virus isolate immunologically related thereto, under conditions which allow the formation of an antigen-antibody complex, said antibodies being fixed to a solid phase,
   b. washing the solid phase,
   c. adding a labeled antibody, and
   d. detecting the antigen-antibody complex.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE,

1. Impfstoff zum Schutz von Schweinen gegen porcines reproduktives Respirationssyndrom (PRRS), der ein ein virales Antigen, welches von dem unter Hinterlegungsnummer I-1140 bei CNCM hinterlegten PRRS-Virus, oder von einem PRRS-Virusisolat, das mit diesem immunologisch verwandt ist, stammt, worin das virale Antigen aus inaktiviertem PRRS-Virus besteht und der Impfstoff umfasst:
   a. Menge des inaktivierten viralen Antigens, welche äquivalent zu einem prä-Inaktivationsvirustiter von mindestens $10^{6.5}$ $TCID_{50}$/ml ist, vorzugsweise mindestens $10^{7.5}TCID_{50}$/ml, oder
   b. ein Adjuvans, ausgewählt aus der Gruppe bestehend aus Vitamin E-Azetat-Ö/W-Emulsion, Aluminiumphosphat, - oxid, eine Öl-Emulsion, vorausgesetzt, die Öl-Emulsion ist nicht Freund'sches Adjuvans, und Saponine, oder
   c. den PRRS-Virus der in CNCM unter der Hinterlegungsnummer I-1140 hinterlegt wurde.

2. Impfstoff zum Schutz von Schweinen gegen porcines reproduktives Respirationssyndrom (PRRS), der ein virales Antigen umfasst, das von einem unter der Hinterlegungsnummer I-1140 in CNCM hinterlegten Virus oder einem Virusisolat, welches immunologisch mit diesem verwandt ist, stammt, worin das

virale Antigen aus lebendem Virus besteht und der Impfstoff umfasst:

a. den PRRS-Virus in lyophilisierter Form oder

b. einen Stabilisator oder

c. eine Menge an $10^{1.0}$-$10^{7.0}$ $TCID_{50}$ des PRRS-Virus pro Dosis oder

d. ein Adjuvans ausgewählt aus der Gruppe bestehend aus Vitamin E-Azetat-O/W-Emulsion, Aluminiumphosphat,-oxid, eine Öl-Emulsion und Saponine oder

e. den PRRS-Virus der unter der Hinterlegungsnummer I-1140 in CNCM hinterlegt wurde.

3. Impfstoff zum Schutz von Schweinen gegen porcines reproduktives Respirationssyndrom (PRRS), der mehr als eine Antigenkomponente umfasst, dadurch gekennzeichnet, dass die erste Komponente, in CNCM unter der Hinterlegungsnummer I-1140 hinterlegte PRRS-Virus oder ein mit diesem immunologisch verwandtes Virusisolat umfasst, und eine weitere Antigenkomponente, die von einem nichtverwandten porcinen Krankheitserreger stammt.

4. Unter Hinterlegungsnummer I-1140 in CNCM hinterlegtes PRRS-Virus oder ein mit diesem immunologisch verwandtes PRRS-Virusisolat, das in Zellkultur zu einem Titer von mindestens $10^{6.0}TCID_{50}$/ml und vorzugsweise bis mindestens $10^{7.0}TCID_{50}$/ml vermehrt werden kann.

5. Biologische Zusammensetzung die den PRRS-Virus gemäss Anspruch 4 umfasst.

6. Verfahren zur Herstellung eines lebenden Impfstoffs zum Schutz von Schweinen gegen PRRS, welches die folgenden Schritte umfasst :

a. 10- 200-faches Passagieren des in CNCM unter Hinterlegungsnummer I-1140 hinterlegten Virus oder eines mit diesem immunologisch verwandten Virusisolates auf einem suszebtiblen Substrat;

b. Vermehren des geschwächten Virus auf einem suszebtiblen Substrat; und

c. Sammeln und Verarbeiten des Virus zu einem Impfstoffpräparat.

7. Verfahren zur Herstellung eines inaktivierten Impfstoffs zum Schutz von Schweinen gegen PRRS, welches den Schritt der Inaktivierung des unter Hinterlegungsnummer I-1140 in CNCM hinterlegten, kultivierten PRRS-Virus oder eines mit diesem immunologisch verwandten Virusisolates umfasst, mit einem inaktivierenden Agens, das aus der Gruppe bestehend aus β-Propiolakton, Ethylenimin oder ein Derivat davon, einem organischen Lösungsmittel, Röntgenstrahlen und γ-Strahlung gewählt wird.

8. Verfahren zur Herstellung von viralem Antigen, das von einem in der CNCM unter Hinterlegungsnummer I-1140 hinterlegten PRRS-Virus oder einem mit diesem immunologisch verwandten Virusisolat stammt, welches die Schritte umfasst:

a. Animpfen suszebtibler Gewebezellen mit dem Virus,

b. Kultivieren der Zellen, und

c. Ernten des viralen Antigens aus der Kultur, wobei der Titer vor der Ernte mindestens $10^{6.0}TCID_{50}$/ml beträgt.

9. Nachweisverfahren für Antikörper gegen PRRS-Virus, welches die Schritte umfasst:

a. Inkubieren einer auf anti-PRRS-Virus-Antikörper verdächtigen Probe mit viralem PRRS-Antigenreagens, das von einem, unter Hinterlegungsnummer I-1140 in der CNCM hinterlegten PRRS-Virus oder einem mit diesem immunlogisch verwandten PRRS-Virusisolat stammt, unter Bedingungen, welche die Bildung eines Antikörper-Antigenkomplexes erlauben und

b. Nachweisen des Antikörper-Antigenkomplexes durch Verwendung eines markierten Antikörpers, wobei Marker aus einer Gruppe bestehend aus Fluoreszenzmarker, Chemilumineszenzmarker, radioaktive Marker, Farbmoleküle und Enzymmarkern gewählt wird, vorausgesetzt, der Enzymmarker wird nicht in einem Immun-Peroxidase-Monolayer-Assay (IPMA) angewendet.

10. Verfahren gemäss Anspruch 9, dadurch gekennzeichnet, dass das virale Antigenreagens ein virales Polypeptid ist.

11. Verfahren gemäss Anspruch 9, dadurch gekennzeichnet, dass das virale Antigenreagens ein Viruspartikel ist.

12. Verfahren gemäss Anspruch 9, dadurch gekennzeichnet, dass das Verfahren aus einem enzymgebundenen Immunabsorbtionsassay (ELISA) besteht.

13. Verfahren gemäss Anspruch 9, dadurch gekennzeichnet, dass das Verfahren aus einem Immunofluoreszenztest (IFT) besteht.

14. Nachweisverfahren für virales PRRS-Antigen in einer Probe, welches die Schritte umfasst:
    a. Inkubieren der Probe mit Antikörpern, die für den unter Hinterlegungsnummer I-1140 in der CNCM hinterlegten PRRS-Virus oder ein mit diesem immunologisch verwandtes PRRS-Virusisolat spezifisch sind, unter Bedingungen, die die Bildung eines Antkörper-Antigenkomplexes erlauben, wobei genannte Antikörper auf einer festen Phase fixiert sind,
    b. Waschen der festen Phase,
    c. Zufügen des markierten Antikörpers und
    d. Nachweisen des Antigen-Antikörper-Komplexes.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Herstellung eines Impfstoffs zum Schutz von Schweinen gegen porcines reproduktives Respirationssyndrom (PRRS), welches ein virales Antigen das von dem in der CNCM unter Hinterlegungsnummer I-1140 hinterlegten PRRS-Virus oder einem mit diesem immunologisch verwandten Virusisolat stammt, worin das virale Antigen inaktivierter PRRS-Virus ist und der Impfstoff umfasst:
    a. eine Menge des inaktivierten viralen Antigens, äquivalent mit einem Präinaktivierungvirustiter von mindestens $10^{6.5}TCID_{50}$/ml, vorzugsweise von mindestens $10^{7.5}TCID_{50}$/ml oder
    b. ein Adjuvans ausgewählt aus der Gruppe bestehend aus Vitamin E-Azetat-Ö/W-Emulsion, Aluminiumphosphat, - oxid, eine Öl-Emulsion, vorausgesetzt, die Öl-Emulsion ist nicht Freund'sches Adjuvans, und Saponine, oder
    c. den PRRS-Virus, der in CNCM unter der Hinterlegungsnummer I-1140 hinterlegt wurde und worin das virale Antigen mit einem pharmazeutisch geeigneten Träger vermischt ist.

2. Verfahren für die Herstellung eines Impfstoffs zum Schutz von Schweinen gegen porcines reproduktives Respirationssyndrom (PRRS), welches ein virales Antigen umfasst, das von dem in der CNCM unter Hinterlegungsnummer I-1140 hinterlegten PRRS-Virus oder einem mit diesem immunologisch verwandten PRRS-Virusisolat stammt, worin das virale Antigen ein lebender PRRS-Virus ist und der Impfstoff umfasst:
    a. den PRRS-Virus in lyophilisierter Form oder
    b. einen Stabilisator oder
    c. eine Menge von $10^{1.0}$-$10^{7.0}TCID_{50}$ des PRRS-Virus pro Dosis
    d. ein Adjuvans ausgewählt aus der Gruppe bestehend aus Vitamin E-Azetat-Ö/W-Emulsion, Aluminiumphosphat, - oxid, eine Öl-Emulsion und Saponine, oder
    e. den PRRS-Virus, der in der CNCM unter der Hinterlegungsnummer I-1140 hinterlegt wurde und worin das virale Antigen mit einem pharmazeutisch geeigneten Träger vermischt ist.

3. Verfahren zur Herstellung eines Impfstoffs zum Schutz von Schweinen gegen porcines reproduktives Respirationssyndrom (PRRS), das mehr als eine Antigenkomponente umfasst, dadurch gekennzeichnet, dass eine erste Komponente das in der CNCM unter Hinterlegungsnummer I-1140 hinterlegte PRRS-Virus oder ein mit diesem immunologisch verwandtes PRRS-Virusisolat umfasst, und eine weitere Antigenkomponente, die von einem nicht verwandten porcinen Krankheitserreger stammt, worin die antigenen Komponenten mit einem pharmazeutisch geeigneten Träger vermischt sind.

4. Verfahren zur Herstellung eines PRRS-Virus, der unter Hinterlegungsnummer I-1140 in der CNCM hinterlegt wurde oder ein mit diesem immunologisch verwandtes PRRS-Virusisolat, wobei der Virus in Zellkultur mindestens zu einem Titer von $10^{6.0}TCID_{50}$/ml und vorzugsweise bis mindestens $10^{7.0}TCID_{50}$/ml vermehrt werden kann, worin eine Zellkultur mit dem Virus infiziert und der Virus nach der Vermehrung aus der Kultur geerntet wird.

5. Verfahren zur Herstellung eines lebenden, abgeschwächten Impfstoffs gegen PRRS, welcher die folgenden Schritte umfasst:

a. 10-200-faches Passagieren des in der CNCM unter Hinterlegungsnummer I-1140 hinterlegten PRRS-Virus oder eines mit diesem immunologisch verwandten PRRS-Virusisolates auf einem suszebtiblen Substrat,
b. Vermehrung des abgeschwächten Virus auf einem suszebtiblen Substrat, und
c. Sammeln und Weiterverarbeiten des Virus zu einem Impfstoffpräparat.

6. Verfahren zur Herstellung eines inaktivierten Impfstoffs zum Schutz von Schweinen gegen PRRS, welches den Schritt der Inaktivierung des unter Hinterlegungsnummer I-1140 in der CNCM hinterlegten, kultivierten PRRS-Virus oder eines mit diesem immunologisch verwandten PRRS-Virusisolates, mit einem Inaktivierungsagens umfasst, das aus der Gruppe bestehend aus β-Propiolakton, Ethylenimin oder ein Derivat davon, ein organisches Lösungsmittel, Röntgenstrahlen und γ-Strahlung ausgewählt wird.

7. Verfahren zur Herstellung von viralem Antigen, das von dem in der CNCM unter Hinterlegungsnummer I-1140 hinterlegten PRRS-Virus oder einem mit diesem immunologisch verwandten PRRS-Virusisolat stammt, welches die Schritte umfasst:
a. Animpfen suszebtibler Gewebezellen mit dem Virus,
b. Kultivieren der Zellen und
c. Ernten des viralen Antigens aus der Kultur, wobei der Titer vor der Ernte mindestens $10^{6.0}TCID_{50}$/ml entspricht.

8. Nachweisverfahren für Antikörper gegen PRRS-Virus, welches die Schritte umfasst:
a. Inkubieren einer auf anti-PRRS-Virusantikörper verdächtigen Probe mit viralem PRRS-Antigenreagens, das von dem unter der Hinterlegungsnummer I-1140 in der CNCM hinterlegten PRRS-Virus oder einem mit diesem immunologisch verwandten PRRS-Virusisolat stammt, unter Bedingungen, welche die Bildung eines Antikörper-Antigenkomplexes erlauben und
b. Nachweisen des Antikörper-Antigenkomplexes durch Verwendung eines markierten Antikörpers, wobei der Marker aus einer Gruppe bestehend aus, Fluoreszenzmarker, Chemilumineszenzmarker, radioaktive Marker, Farbmolekülmarker und Enzymmarkern gewählt wird, vorausgesetzt, der Enzymmarker wird nicht in in einem Immuno-Peroxidase-Monolyer-Assay (IPMA) angewendet.

9. Verfahren gemäss Anspruch 8, dadurch gekennzeichnet, dass das virale Antigenreagens aus einem viralen Polypeptid besteht.

10. Verfahren gemäss Anspruch 8, dadurch gekennzeichnet, dass das virale Antigenreagens aus einem Viruspartikel besteht.

11. Verfahren gemäss Anspruch 8, dadurch gekennzeichnet, dass das Verfahren aus einem enzymgebundenen Immunadsorbtionsassay (ELISA) besteht.

12. Verfahren gemäss Anspruch 8, dadurch gekennzeichnet, dass das Verfahren ein Immunfluoreszenztest (IFT) ist.

13. Verfahren zum Nachweis von viralem PRRS-Antigen in einer Probe, welches die Schritte umfasst:
a. Inkubieren der Probe mit Antikörpern, die für den unter Hinterlegungsnummer I-1140 in CNCM hinterlegten PRRS-Virus oder ein mit diesem immunologisch verwandtes PRRS-Virusisolat spezifisch sind, unter Bedingungen, die die Bildung eines Antkörper-Antigenkomplexes erlauben, wobei genannte Antikörper auf einer festen Phase fixiert sind,
b. Waschen der festen Phase und
c. Zufügen des markierten Antikörpers und
d. Nachweisen des Antigen-Antikörperkomplexes.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE,

1. Un vaccin pour la protection des porcs contre le syndrome respiratoire reproducteur du porc (PRRS) comprenant un antigène viral provenant d'un virus PRRS déposé auprès du CNCM sous le numéro

d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans lequel l'antigène viral est un virus PRRS inactivé et le vaccin comprend:

a) une quantité de l'antigène viral inactivé qui est équivalente à un titre en virus avant inactivation d'au moins $10^{6,5}$ $TCID_{50}$/ml, de préférence d'au moins $10^{7,5}$ $TCID_{50}$/ml, ou

b) un adjuvant sélectionné dans le groupe consistant en émulsion H/E vitamine E acétate, oxyde, phosphate d'aluminium, une émulsion dans l'huile à condition que l'émulsion dans l'huile ne corresponde pas à l'adjuvant de Freund, et saponines, ou

c) le virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140.

2. Un vaccin pour la protection des porcs contre le syndrome respiratoire reproducteur du porc (PRRS) comprenant un antigène viral provenant d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans lequel l'antigène viral est un virus PRRS vivant et le vaccin comprend:

a) le virus PRRS sous une forme lyophilisée, ou

b) un agent stabilisant, ou

c) une quantité de $10^{1,0}$-$10^{7,0}$ $TCID_{50}$ du virus PRRS par dose, ou

d) un adjuvant sélectionné dans le groupe consistant en émulsion H/E vitamine E acétate, oxyde, phosphate d'aluminium, une émulsion dans l'huile et saponines, ou

e) le virus PRRS dépose auprès du CNCM sous le numéro d'accès I-1140.

3. Un vaccin pour la protection des porcs contre le syndrome respiratoire reproducteur du porc (PRRS) comprenant plus d'un composant antigénique, caractérisé en ce qu'un premier composant comprend le virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140, ou un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, et un autre composant antigénique qui provient d'un agent pathogène porcin non apparenté.

4. Un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, lequel virus peut être propagé dans une culture cellulaire à un titre d'au moins $10^{6,5}$ $TCID_{50}$/ml et de préférence d'au moins $10^{7,0}$ $TCID_{50}$/ml.

5. Une composition biologique comprenant le virus PRRS selon la revendication 4.

6. Un procédé de préparation d'un vaccin atténué vivant pour la protection des porcs contre le PRRS, comprenant les étapes de:

a) passage d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, sur un substrat sensible, 10-200 fois.

b) multiplication du virus atténué sur un substrat sensible, et

c) récupération du virus et transformation du virus en une préparation de vaccin.

7. Un procédé de préparation d'un vaccin inactivé pour la protection des porcs contre le PRRS, comprenant l'étape d'inactivation d'un virus PRRS cultivé déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, avec un agent d'inactivation sélectionné dans le coupe consistant en β-propiolactone, éthylène-imine ou un dérivé de ces derniers, un solvant organique, des radiations X et des radiations γ.

8. Un procédé de préparation d'un antigène viral provenant d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, comprenant les étapes de:

a) inoculation de cellules de tissu sensibles avec le virus,

b) culture des cellules, et

c) récolte de l'antigène viral à partir de la culture, de façon à ce que le titre avant la récolte soit d'au moins $10^{6,0}$ $TCID_{50}$/ml.

9. Un procédé de détection des anticorps dirigés contre le virus PRRS, comprenant les étapes de:

a) incubation d'un échantillon à tester dans lequel on suspecte la présence d'anticorps dirigés contre le virus PRRS avec un réactif correspondant à un antigène viral PRRS obtenu à partir d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans des conditions qui permettent la formation d'un

complexe antigène-anticorps, et
b) détection du complexe antigène-anticorps par utilisation d'un anticorps marqué, le marqueur étant sélectionné dans le groupe consistant en marqueurs fluorescents, marqueurs chimio-luminescents, marqueurs radioactifs, molécules de colorant et marqueurs enzymatiques, à condition que le marqueur enzymatique ne soit pas utilisé dans un test immunologique sur monocouche avec la peroxydase (IPMA).

10. Un procédé selon la revendication 9, caractérisé en ce que le réactif antigénique viral est un polypeptide viral.

11. Un procédé selon la revendication 9, caractérisé en ce que le réactif antigénique viral est une particule virale.

12. Un procédé selon la revendication 9, caractérisé en ce que le procédé est un test sur immuno-adsorbant lié à une enzyme (test ELISA).

13. Un procédé selon la revendication 9, caractérisé en ce que le procédé est un test d'immunofluorescence (IFT).

14. Un procédé de détection d'un antigène viral PRRS dans un échantillon à tester, comprenant les étapes de:
a) incubation de l'échantillon à tester avec des anticorps spécifiques d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans des conditions qui permettent la formation d'un complexe antigène-anticorps, lesdits anticorps étant fixes à une phase solide,
b) lavage de la phase solide,
c) addition d'un anticorps marqué, et
d) détection du complexe anticorps-antigène.

Revendications pour les Etats contractants suivants : ES, GR

1. Un procédé de préparation d'un vaccin pour la protection des porcs contre le syndrome respiratoire reproducteur du porc (PRRS) comprenant un antigène viral provenant d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans lequel l'antigène viral est un virus PRRS inactivé et le vaccin comprend:
a) une quantité de l'antigène viral inactivé qui est équivalente à un titre en virus avant inactivation d'au moins $10^{6,5}$ $TCID_{50}$/ml, de préférence d'au moins $10^{7,5}$ $TCID_{50}$/ml, ou
b) un adjuvant sélectionné dans le coupe consistant en émulsion H/E vitamine E acétate, oxyde, phosphate d'aluminium, une émulsion dans l'huile à condition que l'émulsion dans l'huile ne corresponde pas à l'adjuvant de Freund, et saponines, ou
c) le virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140,
et dans lequel l'antigène viral est mélangé avec un support pharmaceutiquement acceptable.

2. Un procédé de préparation d'un vaccin pour la protection des porcs contre le syndrome respiratoire reproducteur du porc (PRRS) comprenant un antigène viral provenant d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans lequel l'antigène viral est un virus PRRS vivant et le vaccin comprend:
a) le virus PRRS sous une forme lyophilisée, ou
b) un agent stabilisant, ou
c) une quantité de $10^{1,0}$-$10^{7,0}$ $TCID_{50}$ du virus PRRS par dose, ou
d) un adjuvant sélectionné dans le groupe consistant en émulsion H/E vitamine E acétate, oxyde, phosphate d'aluminium, une émulsion dans l'huile et saponines, ou
e) le virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140,
et dans lequel l'antigène viral est mélangé avec un support pharmaceutiquement acceptable.

3. Un procédé de préparation d'un vaccin pour la protection des porcs contre le syndrome respiratoire reproducteur du porc (PRRS) comprenant plus d'un composant antigénique, caractérisé en ce qu'un premier composant comprend le virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140,

ou un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, et un autre composant antigénique qui provient d'un agent pathogène porcin non apparenté, et dans lequel les composants antigéniques sont mélangés avec un support pharmaceutiquement acceptable.

4. Un procédé de préparation d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, lequel virus peut être propagé dans une culture cellulaire à un titre d'au moins $10^{6,5}$ $TCID_{50}$/ml et de préférence d'au moins $10^{7,0}$ $TCID_{50}$/ml, dans lequel une culture cellulaire est infectée avec le virus et le virus est récolté à partir de la culture après la propagation.

5. Un procédé de préparation d'un vaccin atténué vivant pour la protection des porcs contre le PRRS, comprenant les étapes de:
   a) passage d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, sur un substrat sensible: 10-200 fois,
   b) multiplication du virus atténué sur un substrat sensible, et
   c) récupération du virus et transformation du virus en une préparation de vaccin.

6. Un procédé de préparation d'un vaccin inactivé pour la protection des porcs contre le PRRS, comprenant l'étape d'inactivation d'un virus PRRS cultivé déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, avec un agent d'inactivation sélectionné dans le groupe consistant en β-propiolactone, éthylène-imine ou un dérivé de ces derniers, un solvant organique, des radiations X et des radiations γ.

7. Un procédé de préparation d'un antigène viral provenant d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'isolat d'un vie PRRS immunologiquement apparenté à cc dernier comprenant les étapes de:
   a) inoculation de cellules de tissu sensibles avec le virus,
   b) culture des cellules, et
   c) récolte de l'antigène viral à partir de la culture, de façon à ce que le titre avant la récolte soit d'au moins $10^{6,0}$ $TCID_{50}$/ml.

8. Un procédé de détection des anticorps dirigés contre le virus PRRS, comprenant les étapes de:
   a) incubation d'un échantillon à tester dans lequel on suspecte la présence d'anticorps dirigés contre le virus PRRS avec un réactif correspondant à un antigène viral PRRS obtenu à partir d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans des conditions qui permettent la formation d'un complexe antigène-anticorps, et
   b) détection du complexe antigène-anticorps par utilisation d'un anticorps marqué, le marqueur étant sélectionné dans le groupe consistant en marqueurs fluorescent, marqueurs chimio-luminescents, marqueurs radioactifs, molécules de colorant et marqueurs enzymatiques, à condition que le marqueur enzymatique ne soit pas utilisé dans un test immunologique sur monocouche avec la peroxydase (IPMA).

9. Un procédé selon la revendication 8, caractérisé en ce que le réactif antigénique viral est un polypeptide viral.

10. Un procédé selon la revendication 8, caractérisé en ce que le réactif antigénique viral est une particule virale.

11. Un procédé selon la revendication 8, caractérisé en ce que le procédé est un test sur immuno-adsorbant lié à une enzyme (test ELISA).

12. Un procédé selon la revendication 8, caractérisé en ce que le procédé est un test d'immunofluorescence (IFT).

13. Un procédé de détection d'un antigène viral PRRS dans un échantillon à tester, comprenant les étapes de:

a) incubation de l'échantillon à tester avec des anticorps spécifiques d'un virus PRRS déposé auprès du CNCM sous le numéro d'accès I-1140 ou d'un isolat d'un virus PRRS immunologiquement apparenté à ce dernier, dans des conditions qui permettent la formation d'un complexe antigène-anticorps, lesdits anticorps étant fixés à une phase solide,
b) lavage de la phase solide,
c) addition d'un anticorps marqué, et
d) détection du complexe anticorps-antigène.

Figure 1